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Vanadate complexes of 3-hydroxy-1,2-dimethyl-pyridinone: Speciation, structure and redox properties

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ABSTRACT

Several articles were published about the vanadate–3-hydroxy-1,2-dimethyl-pyridinone (Hdhp) system, however, the results are contradictory and not complete: pH-potentiometry and ^{51}V NMR spectroscopy were used to clarify this complicated system. The eleven peaks in the spectra at different chemical shifts were assigned to ten stoichiometrically different compounds; four of them are new, never identified or assigned before. Besides the simple mono (in two different protonation states) and bis complexes (in three different protonation states) a tris complex, three dinuclear and a trinuclear complex were found based on the ^{51}V NMR spectra measured at different pH values and various metal ion concentrations and metal-to-ligand ratios. As a joint evaluation of the two methods, overall stability constants were calculated for all species.

X-ray structure of the potassium salt of the bis complex, $[V(V)O_2(dhp)_2]^-$ was also determined. The trans effect of the oxido-oxygens results in maltolato-type coordination of the ligand instead of the catecholate-like chelation.

The redox properties of $[V(V)O_2(dhp)_2]^-$ and some other prodrug vanadium(V) bis complexes were investigated by spectrophotometry in aqueous solution via their reduction by glutathione (GSH) and L-ascorbic acid (ASC) under strictly anaerobic conditions and by cyclic voltammetry at physiological pH. The reduction was found to be much faster by ASC in all cases as compared with GSH and the reaction rate of the reduction of $[V(V)O_2(dhp)_2]^-$ was prominently high most probably due to the formation of the significantly higher stability of the corresponding vanadium(IV) complex.

1. Introduction

Numerous vanadium(IV) and (V) complexes showed significant antidiabetic activity in preclinical *in vitro* and *in vivo* studies [1–4]. One of them the bis(ethylmaltolato)oxovanadium(IV) (BEOV) complex has entered into Phase IIa trial [5]. The active vanadium species exhibit *ca.* 30–70% of the activity of insulin *in vitro*, and generally the efficacy of the V(IV) salt and especially complexes exceeds the originally tested V(V) salts [5, 6]. However the V(V)-compounds tend to be less toxic than V(IV)-complexes, and in general there is no significant correlation between vanadium oxidation state and the insulin-mimetic efficacy [3].

Up to now the most effective hypoglycemic drug candidates are the orally available charge-neutral bis complexes of V(IV) formed with bidentate ligands. The advantage of these metal complexes over the inorganic oxovanadium(IV) salts is their increased bioavailability and thus enhanced pharmaceutical efficacy. According to the stability of this type of complexes, they are usually not stable enough at the pH of the gastric juice resulting in unfavorable uptake, however, that can be bypassed by means of proper drug formulation such as encapsulation methods [7]. Based on the dosage range data of the clinical trials and the absorption properties of BEOV *ca.* 20 μM is estimated as the maximum concentration of vanadium attainable in the human blood serum during the treatment of diabetes mellitus [8]. This kind of vanadium complexes shows facile interconversion between the oxidation states (IV and V) and biologically relevant reducing agents such as L-ascorbic acid (ASC, $10–80 \times 10^{-6}$ mol dm^{-3}), cysteine (33×10^{-6} mol dm^{-3}), glutathione (GSH, 4×10^{-6} mol dm^{-3}), uric acid ($200–400 \times 10^{-6}$ mol dm^{-3}), alpha-tocopherol ($20–30 \times 10^{-6}$ mol dm^{-3}) *etc.* and the dissolved oxygen ensure that both V(IV) and V(V) species are relevant under serum conditions [9].

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It was pointed out in our previous works that vanadium in oxidation state IV and V is bound mostly to serum transferrin in the therapeutically relevant concentration range and the original carrier ligand is displaced completely. The only exception among the bidentate drug candidate ligands is the 3-hydroxy-1,2-dimethyl-pyridinone (Hdhp) where the dissociation of the original complex is not complete in serum and the ligand tends to form V(IV)O–apotransferrin–dhp ternary complexes even at such concentration conditions [4,9,10]. However insulin-mimetic efficacy of the $[V(IV)O(dhp)_2]$ complex looks ordinary [3,11,12].

Several articles were published about the speciation of the vanadate–dhp system [13–16], although the conclusions are sometimes contradictory in the different publications and the final picture is still not complete.

In the first short publication pH-metric results were reported [13] and the following species, mainly mono and bis complexes in different protonation forms were identified: $[(H_2VO_4)(HL)]^- \equiv [VO_2(dhp)OH]^-$, $[H(H_2VO_4)(HL)] \equiv [VO(dhp)(OH)_2]$, $[(H_2VO_4)(HL)_2]^- \equiv [VO_2(dhp)_2]^-$, $[H(H_2VO_4)(HL)_2] \equiv [VO(OH)(dhp)_2]$, $[H_2(H_2VO_4)(HL)_2] \equiv [VO(dhp)_2]^+$. No distinction was made if protonation occurs on the vanadate side or the ligand side of the complex. Only two ^{51}V NMR peaks (mono complexes: $\delta(^{51}V) = -502$ ppm, bis complexes: $\delta(^{51}V) = -476$ ppm) were identified at the physiological pH. Based on cyclic voltammetric (CV) measurements the authors also concluded that the reduction of the vanadate complexes to oxovanadium(IV) is not reversible, but the irreversibility is less pronounced at pH 3 [13].

Later X-ray structure for a trinuclear complex $[(VO_2)_3(dhp)_3 \cdot H_2O]$ has been published [14]. In this cyclic compound, μ-oxygens form bridges between the vanadium centres, the three ligands and the vanadates are not equivalent due to an extra ligand-O–V bound.

The ^{51}V NMR spectral work in the same publication [14] is more detailed, but is still not complete: it reports unidentified species (C: $\delta(^{51}V) = -420 - -405$ ppm; C′: $\delta(^{51}V) = -350$ ppm). A peak at $\delta^{51}V = -489$ ppm was assigned improperly to a trinuclear complex $[(VO_2)_3(dhp)_3]$ instead of mononuclear one $[(VO_2)(dhp)]$). ^1H NMR measurements for the mono and bis complexes have also been published [14], but no thermodynamic information was concluded from these measurements.

In the third article [15] stability and protonation constants were determined from the ^{51}V NMR data: the stability constants for the mono and bis complexes significantly differ from the earlier published values [13]. The authors determined one protonation constant (pK) for the mono and two others for the bis complexes by ^{51}V NMR spectroscopy. These processes were supposed to occur on the metal side. However, based on ^1H NMR measurements further protonation processes for both the mono and bis complexes were assumed which should occur on the ligand side [15].

From methanol–water solvent mixture a dinuclear dhp–vanadate–methyl–ester complex was ($[VO(OMe)(dhp)]_2O$) prepared, in which beside two oxygen donor atoms of each dhp ligand a μ-oxygen also forms a bridge between the vanadium centres. ^1H and ^{51}V NMR methods have been used to determine the equilibrium in different methanol-water mixtures.

Addition of methanol to the system makes the speciation even more complicated because the alcohol is able to react with the V-OH part of the complexes forming "esters", and this process is not advantageous for understanding the feature of the basic V(V)–dhp system. Temperature dependence of ^{51}V NMR spectra and 2D ^1H homonuclear NMR spectra was also measured and isomers of the dinuclear ($[V(V)O(OMe)(dhp)]_2O$) and the bis complex $[V(V)O(OMe)(dhp)_2]$ were detected [16].

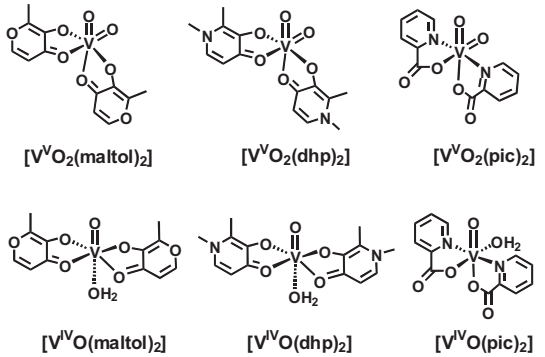
In order to clarify the speciation in the V(V)–dhp system and identify the composition and the stability of the complexes pH-metric and ^{51}V NMR spectroscopic measurements were used in the present work.

Vanadium may be assumed to enter the cells via the transferrin receptors, when V(IV), or via the phosphate or sulfate pathway, when V(V) [1]. In the intracellular medium V(V) species may suffer reduction by certain cell components and ASC and GSH are the most important and abundant cellular antioxidants (0.01–0.02 mol dm^{-3}, 0.5–10 mmol dm^{-3}, respectively) and their role is frequently discussed [17,18]. It is noteworthy that GSH and its oxidized form glutathione disulfide (GSSG) are also able to form binary or ternary $V(V)O_2^+$ and $V(IV)O^{2+}$ complexes by the complete or partial substitution of the carrier ligand [19] and a similar behavior cannot be completely ruled out either for ASC, however based on the conditions and stability ratios the original bis complexes should dominate in the solution before and after the reduction too.

The reaction rate of the reduction of the V(V) complexes basically depends on the type of the reducing agent and that of the coordinating ligand. The greater ability of the chelating ligand to stabilize the V(V) oxidation state, the stronger the tendency to promote the reduction. It was found *e.g.* that NADPH can reduce *in vitro* V(V) complexes having formation constant ($\log K'$) higher than 7 in the case of a series of amino acid, oligopeptide, aminopolycarboxylate ligands [1]. Simple thiols, as other possible reductants, are able to form stable complexes with V(V) at neutral or alkaline pH, however, they are oxidized by the metal ion under other conditions, such as low pH and high thiol excess [1].

Song et al. performed a detailed kinetic study of the reduction of $[V(V)O_2(maltol)_2]$ and $[V(V)O_2(ethylmaltol)_2]$ with ASC or GSH in aqueous solution monitored by UV–Vis spectrophotometric, EPR and ^{51}V NMR techniques [17]. These V(V) complexes showed similar behavior; therefore, the replacement of the methyl group by the ethyl group in the ligand structure had little influence on the reaction rate. The reduction by GSH was found to be much slower than by ASC at physiologically relevant pH. First-order kinetics at large excess of GSH and ASC is suggested and the observed first-order rate constants showed a linear relationship with the concentration of the reductants. An acid dependent mechanism was proposed from kinetic studies with varying pH and carrier ligand concentration.

The reaction mechanism, however, seems to be quite complicated due to the additional binary and ternary complex formation reactions with the reducing agents (*vide supra*). In this work we attempt to compare the effect of the dhp with that of various other carrier ligands on the reduction reaction rate when GSH and ASC are applied as reductants under strictly anaerobic conditions at pH 7.4 via monitoring the spectral changes in the near UV–Vis range. Additionally, cyclic voltammetic investigations were performed on the direct redox processes of the vanadium complexes



Scheme 1. Bis-ligand vanadium(V/IV) complexes formed with maltol = 3-hydroxy-2-methyl-4H-pyran-4-one; dhp = 3-hydroxy-1,2-dimethyl-pyridinone and pic = picolinic acid.